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COMPARATIVE STUDIES OF THE DEVELOPMENT OF TWO SPECIES OF SPOTTED CUTWORM *XESTIA ADELA* AND *XESTIA DOLOSA* (LEPIDOPTERA: NOCTUIDAE), AND IDENTIFICATION OF LARVAE BY ELECTROPHORESIS

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Abstract.—The immature states of *X. adela* Franclemont and *X. dolosa* Franclemont are structurally indistinguishable except for size. In laboratory rearings the average maximum weight of the last larval instar of *X. dolosa* was about double that of *X. adela*; the average weight of *X. dolosa* pupae was similarly greater than that of *X. adela*. The difference in size, measured as the width of the larval head capsules, increased after the fourth instar; at this time the head capsule of *X. dolosa* became distinctively darker. Eight enzymes were surveyed by electrophoresis; two were found to completely distinguish larvae, pupae, and adults of the two species in sympatric populations.

Xestia adela Franclemont and *Xestia dolosa* Franclemont are recently recognized sibling species (Franclemont, 1980) of what was previously known in North America as the spotted cutworm, *Xestia c-nigrum* (L.). As such they are reported to have caused economic damage in localized outbreaks (Beirne, 1971). *Xestia adela* is the more widely distributed, occurring in the northern and central United States and throughout most of Canada, whereas *X. dolosa* is restricted to the northeastern and central United States and southeastern Canada (Franclemont, 1980). In the east, where the two species occur sympatrically, it is not known if accounts of damage by *X. c-nigrum* can be attributed to one or both species.

Adults of *X. adela* and *X. dolosa* can usually be distinguished on the basis of characters of the genitalia and reproductive system (Franclemont, 1980; Hudson, 1981), and by selected measurements of the head and wings; they can also be distinguished electrophoretically by allozymes of adenylate kinase (Hudson and Lefkovitch, 1980).

The use of allozymes (alleles at a single locus) as species diagnostic characters was described by Ayala and Powell (1972) for distinguishing sibling species of *Drosophila*, and these authors established criteria for selecting loci which could be safely considered to be diagnostic. Since then, electrophoretic keys have been constructed for the identification of adults and larvae of several groups of insects that are difficult to identify by structural characters (e.g., Miles, 1979; Berlocher, 1980).

The immature stages of *X. adela* and *X. dolosa* are structurally indistinguishable, except in terms of maximum size. As *Amathes c-nigrum* the larvae have been

Table 1. Maximum weights reached by larvae and pupae of *X. adela* and *X. dolosa* under two different temperature and photoperiod regimes.

Temp.	Photoperiod L:D	No. in Sample	Species	Mean Max. Weight of Larvae (Range)	Mean Max. Weight of Pupae (Range)
24°C	16:8	29	*X. adela*	0.564 (0.442–0.640)	0.281 (0.245–0.332)
		30	*X. dolosa*	0.996 (0.648–1.118)	0.427 (0.367–0.534)
21°C	12:12	25	*X. adela*	0.531 (0.432–0.701)	0.266 (0.177–0.329)
		24	*X. dolosa*	1.032 (0.726–1.222)	0.475 (0.353–0.552)

described as non-specific feeders on vegetable and cereal crops and tobacco; they also climb to feed on fruit and shade trees (Rings, 1977; Rings and Johnson, 1977), but it is not known if this behaviour is typical of both sibling species.

The present study compares the development of *X. adela* and *X. dolosa*, reared under controlled conditions, and applies the method of electrophoresis to the immature stages, in a survey for diagnostic enzyme loci that could be used to identify them in sympatric populations.

Material and Methods

Gravid females of the spring broods of *X. adela* and *X. dolosa* were collected from light traps set up in North Gower and Harrow, Ontario. They were placed in cages for oviposition and eggs were laid on strands of frayed nylon cord, or squares of nylon screening.

Two methods of rearing were employed to provide samples for comparative studies of development (duration of larval instars, head capsule widths, maximum weights) and for electrophoresis.

Method 1 was carried out under two regimes of temperature and photoperiod; 24°C with 16 h light and 8 h dark, and 21°C with 12 h light and 12 h dark.

Fertile eggs were removed from the cages and placed in 3 cm diameter clear polystyrene vials containing small slices of artificial diet spaced between strips of absorptive paper (Hinks and Byers, 1976). Larvae that emerged during a 12 hour period were placed individually in 4.5 × 2.3 × 2.0 cm clear polystyrene boxes containing diet and reared individually through to pupation. The food was changed daily during the early instars and then on alternate days. The vials were examined daily for head capsules which were removed and measured, and the date of each moult was recorded. After reaching the last instar the larvae were weighed daily and the maximum weight attained by each was recorded; this marked the onset of the prepupal period. Prepupae were placed in moistened peat moss until pupation. When the pupal cuticle had hardened the pupae were weighed and sexed and returned to the peat moss until emergence.

Method 2 was carried out at 24°C 16L:8D. The progeny of ten *X. adela* and 5 *X. dolosa* females were reared as single lines. Fifty eggs from each female were

Table 2. Number of days spent at each developmental stage by *X. adela* and *X. dolosa* under two different regimes of temperature and photoperiod (mean values taken from 25–35 insects ±SE)

Species	24°C 16L:8D				21°C 12L:12D			
	Larvae	Prepupae	Pupae	Egg to Adult	Larvae	Prepupae	Pupae	Egg to Adult
X. adela	16.65 ± 0.19	4.35 ± 0.24	12.15 ± 0.13	32.37 ± 0.21	26.64 ± 0.21	4.24 ± 0.13	14.52 ± 0.59	45.17 ± 0.40
X. dolosa	18.20 ± 0.20	9.40 ± 0.26	15.80 ± 0.24	43.43 ± 0.54	35.02 ± 0.24	8.54 ± 0.26	21.26 ± 0.59	64.61 ± 0.40

frozen at −80°C; the remaining eggs from a single female were placed in a vial and after hatching two groups of 10 first instar larvae were removed and frozen. The remaining larvae from each line were maintained separately in larger containers for further development; from each line samples of five larvae of successive instars, as well as newly emerged adults, were removed and frozen. These samples were used for electrophoresis.

Electrophoresis.—The horizontal starch gel method described by Ayala et al. (1972) was employed using 12% by weight of electrostarch (Electrostarch Co., Wisconsin). Eight enzyme loci were surveyed using three different buffer systems. (1) Bridge buffer 0.135 M tris–0.04 M citric acid–0.001 M EDTA pH 7.2, gel buffer diluted 1:2, for adenylate dinase (Adk), α-glycerophosphate (α-Gpdh) and isocitrate dehydrogenase (Idh). (2) Bridge buffer 0.3 M boric acid–0.06 M NaOH pH 8.1, gel buffer 0.08 M tris–0.006 M citric acid pH 8.85 (Poulik, 1957) for hexokinase (Hk), phosphoglucose isomerase (Pgi) and glutamate-oxaloacetate transaminase (Got). (3) Bridge buffer 0.05 M Tris–0.05 M NaH_2PO_4, gel buffer diluted to 1 in 10 (Harris and Hopkinson, 1976) for mannose phosphate isomerase (Mpi).

Results and Discussion

Salkeld (unpublished) has found that the eggs of *X. adela* and *X. dolosa* resemble each other closely in size and chorionic patterns, but that the number of primary (rosette) cells is usually greater in *X. dolosa*. Under the standard rearing conditions (24°C, 16L:8D) fertile eggs of both species darkened within 6–8 days.

The larvae of the two species appear to be structurally indistinguishable except for a difference in size which becomes increasingly evident from the fourth instar. Under two different rearing conditions the final instar mean maximum weight attained by *X. dolosa* larvae was close to double that of *X. adela* (Table 1); the range of maximum weights of individuals in each species was large, but there was no overlap. There was a gradual reduction in size during the prepupal stage by each species, to about one half the maximum larval weight (Table 1).

The total times taken for development by each species from egg to adult, under the two different temperature and photoperiod regimes, differed by 12.8 days for *X. adela* and 21.2 days for *X. dolosa* (Table 2). At 24°C, 16L:8D *X. dolosa* took 11.1 days longer than *X. adela* to complete development, mainly due to longer duration of the prepupal and pupal stages. At 21°C, 12L:12D *X. dolosa* took 19.4 days longer than *X. adela*, but under these conditions the time spent as larvae increased also.

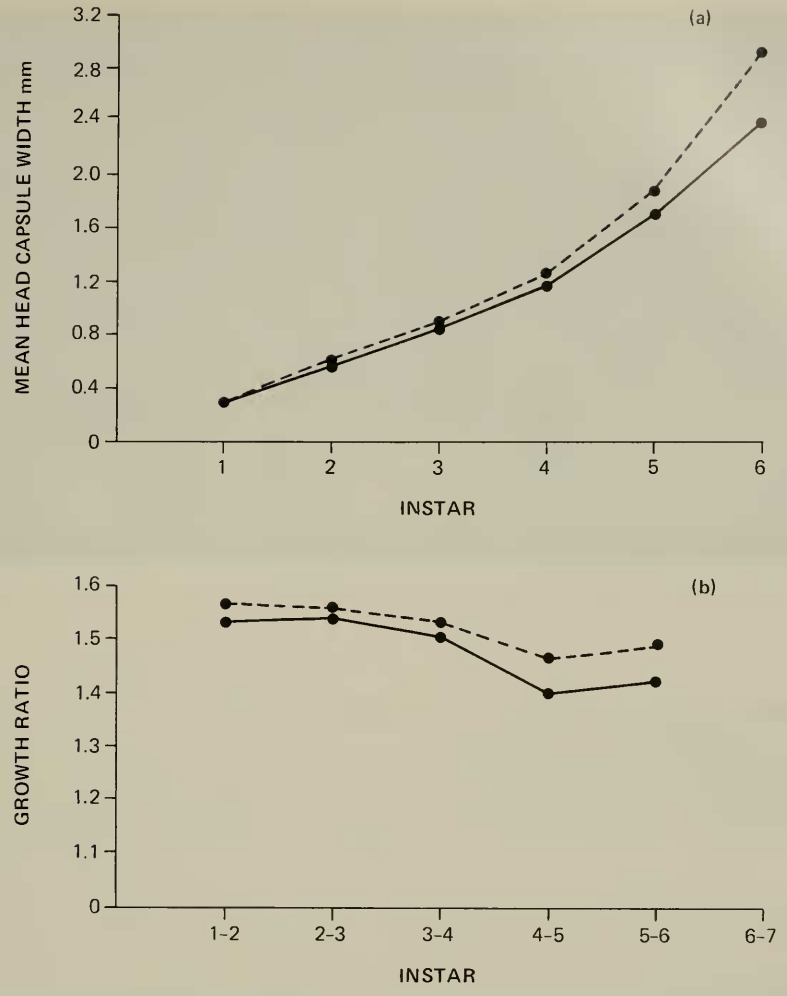

Fig. 1. a, Growth of head width of 1st- to 6th-instar larvae of *Xestia adela* (solid line) and *X. dolosa* (broken line). b, Growth ratios for 1st–6th instars as above.

Comparisons of larval growth in the two species were made in terms of head capsule widths. Little difference was evident between them until the fourth instar (Fig. 1a) when the mean width for *X. adela* was 1.19 mm ± 0.019 (84 individuals), and for *X. dolosa* 1.33 ± 0.023 mm (81). After the fourth instar the difference in size between the species increased at each moult until at the sixth instar (prepupa) the mean head width of *X. adela* larvae was 2.40 ± 0.033 mm (55) and of *X. dolosa* larvae 2.90 ± 0.02 mm (54). Growth ratios (the ratio of the means for two successive instars) are shown in Fig. 1b, and follow a similar pattern in both species. The ratios for instars 1–2, 2–3, and 3–4 were highest, dropping slightly for instar 4–5 and 5–6. The mean growth ratio for *X. adela* was 1.48 and for *X. dolosa* it was 1.52. A small number of the insects reared at 21°C, 12L:12D underwent an additional moult and the resulting adults from these larvae had slightly larger head widths. After the fourth instar the head capsules of *X. dolosa* became

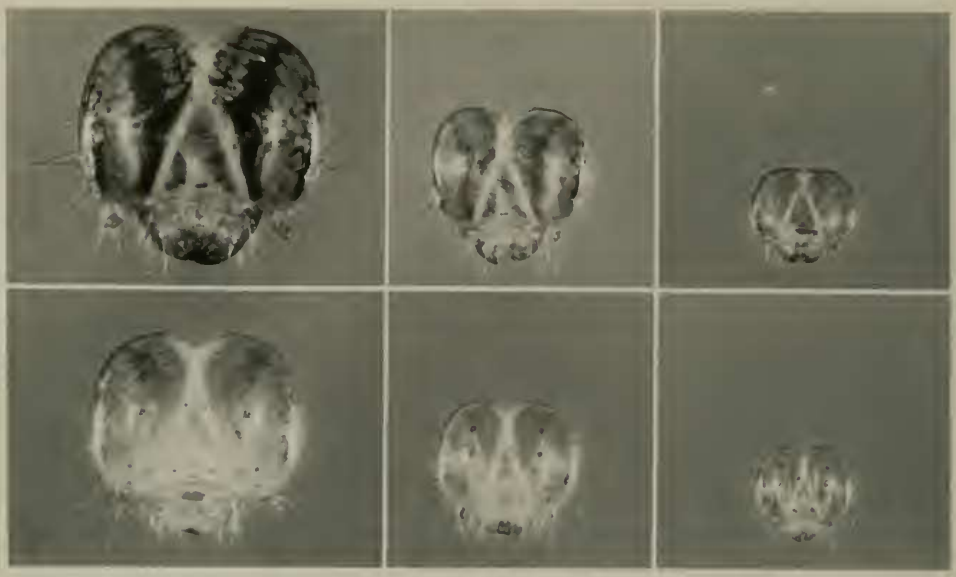

Fig. 2. Head capsules of *Xestia dolosa* (top) and *X. adela* (bottom) larvae. Right to left, 4th–6th instars.

more heavily pigmented than those of *X. adela* (Fig. 2) and individuals could usually be distinguished in this way.

Electrophoresis.—All the enzymes surveyed in the larval stages had been examined previously in field populations of adults, and allele frequencies and segregation data of the polymorphic enzymes in the species have been reported (Hudson and Lefkovitch, 1982). In the present study we have surveyed eight enzymes and selected, for the purpose of identification, those which are diagnostic either because they occur as single banded isoenzymes with different electrophoretic mobilities (i.e., are monomorphic), or because although polymorphic, there are certain alleles that can be used to identify the species with a probability greater than 99.9%.

The enzyme Idh was monomorphic, with a different band characterizing each species. It can be used to identify immatures in Ontario populations by Idh allele 1.0 which typifies *X. adela* and Idh 1.1 which typifies *X. dolosa* (Fig. 3a). The same band distinguished adults in which extracts of the abdomens stained intensely, but the thoraces only faintly. Adk was polymorphic with four alleles (0.94, 0.96, 1.0, 1.03) seen in the larval stages. Adk allele 1.0 was characteristic of *X. adela* and Adk 0.96 of *X. dolosa* (Fig. 3b). This Adk locus was used to identify moths in collections from four locations in Ontario and in a collection of *X. c-nigrum* made in Oxford, England. In the latter collection Adk allele 1.0 was found in 55% of the individuals and 0.96 in none (Hudson and Lefkovitch, 1982). The remaining enzymes were either monomorphic for the same band in both species (α-Gpdh and Got), or polymorphic (Hk, Mpi, Pgi, Pgm) with the same alleles as those found in the previous study of adult moths, and so are not useful for direct identification of individuals. Only larvae of the spring broods were

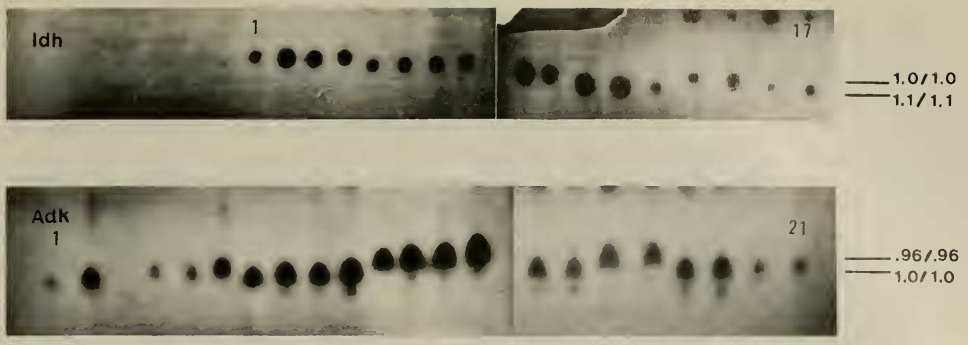

Fig. 3. Diagnostic enzymes isocitrate dehydrogenase (Idh) and adenylate kinase (Adk) in the larvae and adults of *Xestia adela* and *X. dolosa*. Idh: left to right, 1–4, *X. adela* 4th–6th (prepupa) instars; 5–8, *X. dolosa* 4th–6th (prepupa) instars; 9–10, *X. adela* pupae; 11–13, *X. dolosa* pupae (13 = pharate adult); 14–15, *X. adela* newly emerged adults (♂, ♀); 16–17, *X. dolosa* newly emerged adults (♂, ♀). Adk: left to right, 1–2, *X. adela* 2nd and 3rd instars; 3–5, *X. dolosa* 1st–3rd instars; 6–9, *X. adela* 4th–6th (prepupa) instars; 10–13, *X. dolosa* 4th–6th (prepupa) instars; 14–15, *X. adela* pupae; 16–17, *X. dolosa* pupae; 18–19, *X. adela* newly emerged adults (♂, ♀); 20–21, *X. adela* 14 day old adults (♂, ♀).

examined but on the basis of adult studies it is probable that summer brood larvae can be distinguished in the same way.

The existence of diagnostic enzymes provides a direct and definitive method of identifying field collected larvae associated with crop damage by the "spotted cutworm," and to provide information on host preference and feeding behaviour of these sibling species. It is possible also that the difference in the intensity of head capsule pigmentation, seen in our samples, could be used to identify larvae of these species in the field, provided its validity within large sympatric populations is first confirmed by electrophoresis.

The later appearance of *X. dolosa* compared to *X. adela* in both spring and summer broods in Ontario is predictable from the differing lengths of time required to complete development. Hinks and Byers (1976) showed that the length of time taken to reach maturity by different species of *Euxoa* was not related to size and this seems to be true also for these species of *Xestia*. The results of the laboratory rearings also show that the effect on growth of lower temperature and shorter photoperiod, as indicated by the relative extensions of duration of development, is greater in *X. dolosa* than in *X. adela* and may account for the lower number of second field generation *X. dolosa* adults collected at the northern limits of its distribution.

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